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Art. III.—*Sensitising Powers of Proteins of Parasites as tested by the isolated sensitized uterus reaction.*

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Introduction.

This work was carried out for the purpose of investigating the possibility of using the specific sensitiveness of the anaphylactic guinea-pig's isolated uterus as a means of identifying metazoan parasites. The extraordinary sensitiveness of the smooth muscle of anaphylactic guinea pigs, combined with the exquisite specificity of the reaction, as observed in the case of the more common proteins, suggested that, should the same phenomena be met with in the case of proteins of metazoan parasites, one might well apply the method to the specific identification of parasite proteins.

During the course of the work the sensitizing powers of the following parasites were investigated:—*Ascaris equi, Ascaris suilla* and *Toxascaris limbata,* and *Onchocerca gibsoni* as members of the Nematoda; *Fasciola hepatica* as a member of the Trematoda; and *Gastrophilus haemorrhoidalis, G. nasalis* and *G. intestinalis* as members of the Arthropoda.

In addition it was necessary to determine the action on the uterus of a few substances which were needed as preservatives, the following being investigated: Toluol, Chloroform, Phenol and Glycerine.

A few experiments were performed with common proteins, and these serve to demonstrate the phenomena of anaphylactic response and desensitization.

Review of Literature, etc.

The first use of the anaphylactic response of isolated organs was made by Schultz (1). He experimented with sera on isolated guinea pig small intestine and found that a much greater contraction of the smooth muscle occurred when this came from an anaphylactic guinea pig than when it came from a normal pig, the same concentration of serum being used in each case.

This result was greatly extended by Dale (2), who, using the isolated guinea pig uterus horns, was able to demonstrate extremely delicate sensitiveness to the serum proteins used for

sensitising, often obtaining a definite anaphylactic response at a concentration of one in a million of the sera used, and in one case, at a concentration of 1 : 6,500,000 of dry globulin (3); and, when using the purified crystallised egg albumins of the hen and duck, the remarkable result was obtained, that an anaphylactic response occurred at a concentration of 1 : 500 millions! It appears to be quite an established fact that the degree of sensitiveness is a function of the degree of purity of the antigen and its freedom from other proteins. Dale (3) found that, when multi-sensitization was attempted, using simultaneous inoculations of egg white, horse serum and sheep serum, less clear results were obtained after 19 days, and he concluded that a guinea pig receiving small simultaneous injections of several different proteins acquires to none of them the high degree of sensitiveness which, after the same period, might be expected as the result of injecting any one of them singly. He suggested that possibly a more extended incubation period might be necessary under such conditions. He also observed that it appeared that desensitization to one antigen was not wholly without effect on the sensitiveness to the others, though the results he obtained in the few guinea pigs tested showed that there was some degree of independence. He was also able to demonstrate passive sensitiveness in guinea pigs which had received 5 c.c. of immune or anaphylactic serum.

Regarding the specificity of the isolated guinea pig uterus reaction, he demonstrated a high degree of such in an experiment described in (2). A guinea pig received 1/400 c.c. of horse serum fourteen days previously, and the horns were suspended in a 250 c.c. bath. The following sera were added in 0·1 c.c. amounts successively without effect — sheep, cat, rabbit, dog, man—and also egg white in the same dose. However, on adding 0·1 c.c. of horse serum, an immediate maximal contraction of the muscle occurred. The acme of specificity reactions is described by him (4) in an experiment with duck egg albumin. Dale's results have been confirmed many times; among others, by Nathorff (5), who demonstrated the irritant action of fresh serum, anaphylaxis towards sheep serum, specificity between sheep and horse serum, the phenomena of desensitization ("Anti-anaphylaxie") and multisensitization, using sheep and horse serum. He found that guinea pigs sensitized with a mixture of equal parts of these two sera showed a much greater sensitiveness to sheep serum than to horse serum, no matter in which order they were added to the testing bath. The class of substances which are able to bring about the anaphylactic phenomenon in guinea pigs is that known as the antigens, which are typically the native proteins. Anaphylactogenic properties have been demonstrated for proteins of various origins. Osborne and Wells (6) have demonstrated typical anaphylaxis (*in vivo*) to the crystallized albumin from white of egg and to the crystallized protein from various seeds.

Hartley and Dale (7) observed it with a very highly purified sample of crystallized albumin from horse serum. Any denaturing influence rapidly destroys the antigenic efficiency—e.g., boiling of coagulable proteins, digestion. Ten Broek (8) has shown that racemization of a protein by gentle warming with alkali, completely destroys its antigenic activity; Max Burger (9) also showed similar results. He concluded that amino-acids, pure albumoses, protamines, and acid-albumin are not able to sensitize and that hence all preparations which are prepared by acid hydrolysis, peptic and tryptic digestion from plant and animal protein are unsuitable.

The specificity is also demonstrated by the fact that Wells (10) was able to obtain separate and more or less specific anaphylaxis in the guinea pig with each of the five proteins obtained from hen's egg. Also Wells and Osborne (11) using whole animals, showed that the four proteins of milk, namely, casein, lactalbumin, lactoglobulin and an alcohol-soluble protein, are all immunologically distinct. The globulin sensitized to ox serum and caused a reaction in animals sensitized to ox serum. Also Hartley and Dale (6) found that anaphylaxis discriminated between the three proteins separable from horse serum. They also note that sensitiveness to euglobulin appears first (8-10 days), and that to albumin after about twice as long (16-20 days). There was even clear, though less rigid (? difficulty of purification) distinction between the so-called eu- and pseudo-globulins.

It will thus be seen that the anaphylactic test, and in particular the isolated uterus reaction, has immense possibilities as an agent for demonstrating structural or absolute identity of proteins, and for showing on the other hand differences between proteins which by biochemical methods are very difficult to distinguish. It should be noted, however, that Kritchevsky (12) states that rabbits immunised with the red blood cells of the hen produce anaphylactic antibodies against the red blood cells of the sheep. Nevertheless, we confine ourselves to the guinea pig, and use the isolated uterus test, which appears to be strictly specific.

A few authors have appreciated the significance of the above related facts, and attempts have been made to use the anaphylactic reaction as a diagnostic agent. Thomsen (13) and Uhlenhuth (14) and others have shown that the test can be used as a forensic test for blood spots—by sensitizing guinea pigs with solutions of blood spots, and later testing them (*in vivo*) with a second injection of the suspected protein, but they do not consider the method as reliable as the precipitin test. Yamanouchi (15) has attempted to apply the method to the diagnosis of tuberculosis by attempting to passively sensitize guinea pigs with serum of tuberculosis patients and later testing the animals with tuberculin and he claims some positive results, but this has not been confirmed by others. Pfeiffer (16) and Pfeiffer and Finsterer (17) report positive results in the diagnosis of malignant disease by injecting guinea pigs with press-juices of tumours 48 hours after they

received the serum of carcinomatous patients, while these did not occur when normal human serum was used. This has, however, been denied by Ranzi (18). Rhein (19) has shown that the *in vivo* anaphylactic reaction will distinguish between normal urines of various animals, even between those of closely related species, e.g., goat and sheep, and man and ape. Antigenic character of normal human urine was lost by boiling for one hour, or allowing to putrefy for one month, and drying for one month.

In 1912 Guido Guerrini (20) obtained specific anaphylactic reactions by using "nucleoproteins" extracted from various sources—Dog's spleen, Dog's liver, Horse's spleen, Dog serum, *Vibrio cholerae, B. pestis, Fasciola hepatica* and *Dicroecoelium lanceolatum.* Nucleo-proteids were obtained by acid precipitation from alkali extracts of minced tissues (or from bacteria). Organs were perfused with normal saline in an endeavour to remove all traces of blood and serum proteins. It is doubtful whether this is possible, however, in the light of some perfusion experiments performed by Larson and Bell (21), who showed by means of india ink, that only a part of the capillary system is washed out, and who, while not denying the cellular theory of anaphylaxis, point out that Dale's (2) assumption of the bloodlessness of the perfused isolated guinea pig uterus in his experiments is unsound. However, the tissue was then minced and shaken for 24 hours in ice-chest with 20 volumes of 1% aqueous solution of potassium hydroxide. It was allowed to stand, and the supernatant fluid poured off and centrifuged. Then 1% aqueous solution of acetic acid was added, and a precipitate of "nucleoprotein" came down, which was collected on filter paper and dried in vacuo. No attempt to further purify was made. Bacteria were grown in large Petrie dishes, and similarly treated. Parasites were ground in a mortar after washing and similarly treated. Guinea pigs were sensitized by intraperitoneal and subcutaneous inoculation of the following nucleoproteins in 1% solution in 1% aqueous solution of sodium carbonate and tested after 18-28 days.

Dog's spleen nucleoproteid.
Dog's liver ,,
Horse's liver ,,
Horse's spleen ,,
B. pestis ,,
Vibrio cholerae ,,

The second inoculation was made with the other nucleoproteids and specific reactions were obtained with the anaphylactogen.

No experiments were tried of sensitizing guinea pigs with the Helminthic nucleoproteids and of endeavouring to produce distinct specificity between the two which were used.

Another interesting application of the test was by Fellmer (22), who worked with protein extracts of certain Fungi. Among the methods of differentiation used was the anaphylactic (*in vivo*)

method, and he concluded that animals can be made anaphylactic by Fungus-protein. The reaction body is transferable to other animals with the serum, and brings on passive anaphylaxis. The anaphylactic bodies are specific.

Though the present work does not deal with bacterial anaphylaxis, it is interesting to note that Smith (23) was able to obtain an anaphylactic response in 7 cases out of 8 of renal tuberculosis, when the isolated uteri of tuberculous guinea pigs were tested with the addition of 3-5 c.c. of urine from these cases, with partial desensitization after shocking. He says that the capacity for specific contraction is markedly diminished or absolutely abolished after a single maximal response. He used urines in which acid-fast bacilli after proved to be *B. tuberculosis* were found, and included in the same testing bath a normal uterus horn as a control.

Among the other publications which have come under our notice bearing on this year's work with Helminth proteins, is one by Shimamura and Fujii (24), obtained only lately, on a highly toxic substance (Askaron), obtained from *Ascaris lumbricoides* (from man and from swine), and *A. megalocephala* (horse). They state that this same substance, which they believe to belong to the Albumose-peptone group, has a fairly wide distribution, and occurs also in *Filaria immitis*, *Gastrophilus* larvae, *Strongylus vulgare*, *Oxyuris curvula*, and *Trichocephalus depressiusculus*. They differentiate the symptoms of Askaron poisoning from Anaphylactic shock by the following points, though the symptoms are very similar to those of anaphylactic poisoning.

(1) Askaron is primarily toxic without previous sensitization.

(2) Also, since the guinea pig is not a natural host of the above parasites, the presence of antibodies against the body substance of the Helminths (mentioned) is not conceivable, and yet Askaron can give rise to a very severe intoxication in guinea pigs.

(3) Askaron, in sufficient amount, can produce poisoning without exception in horses, dogs, rabbits and guinea pigs and in the latter, the individual susceptibility is so constant that the lethal dose is always constant.

(4) Since the absorption of coagulable protein-material from the intestinal canal under normal conditions is not conceivable, the supposition that protein-material of gastro-intestinal parasites can give rise to a constant hypersensitiveness in their hosts is untenable.

(5) Askaron is a substance of Albumose-peptone character, which is not regarded as a good anaphylactogen.

They treated a series of guinea pigs intraperitoneally with 2-5 c.c. of normal horse blood, and after 24 hours injected 1/5th to 1/8th of the M.L.D. of Askaron intravenously. No alteration of the toxicity was shown, showing that normal horse blood does not contain anaphylatoxin against Ascaris, and that Askaron is primarily toxic.

Also no increase in the toxicity of "crude Askaron" (one of the preparations used) or of defatted Ascaris powder was observed when guinea pigs were prepared with a subcutaneous inoculation of ½-1/5th of the M.L.D., and after 14 days' interval, were tested intravenously with ½-¼ of the lethal dose, i.e., no anaphylactic sensitization occurred.

The literature on anaphylaxis towards parasites is, in general, poor; but the best known example of such anaphylactic reaction is that of the characteristic syndrome following the accidental puncture of an *Echinococcus* cyst during the attempted removal of the same, concerning which there is quite an extensive literature.

The conception that parasites can give rise to anaphylactic symptoms in their hosts under certain conditions does not appear to have been further extended till 1916, when Hadwen (26) published a preliminary note on Hypodermal Anaphylaxis. In 1917, Hadwen and Bruce (27) published their paper on "Anaphylaxis in cattle and sheep, produced by the larvae of *Hypoderma bovis, H. lineata* and *Oestrus ovis*," in which they described serious symptoms and death following the crushing of larvae of *Hypoderma* (the warble fly), which were under the skin of cattle or following intravenous inoculation of aqueous extracts of the same into infested cattle. They also performed similar experiments with sheep infested with larvae of *Oestrus ovis*, the sheep Nasal Bot. They produced probable anaphylactic symptoms in rabbits and guinea pigs with their extracts.

Seyderhelm (28) has tried to explain Equine Pernicious Anaemia as a chronic intoxication with Oestrin, a toxic substance occurring in *Gastrophilus* larvae. In 1916, Favero (29) expressed the opinion that the intoxication described by them following intravenous inoculations of extracts of *Gastrophilus*, was a merely anaphylactic phenomenon.

It was these two latter papers which stimulated Van Es and Schalk (30) to examine the question. They showed, in well-controlled experiments, that *Gastrophilus* larvae living in the stomach of the Horse, could produce sensitization of the animal, with anaphylactic shock following on intravenous inoculation of extracts of such in such amounts as did not affect uninfested horses. From this as starting point they dealt with *Ascaris megalocephala, Trichodectes parumpilosus, Toxascaris limbata*, and *Belascaris marginata, Dipylidium caninum, Taenia serrata, Gyropus ovalis* and *Gyropus porcelli*.

In 1922 appeared Cameron's paper (25) on "Bot" anaphylaxis, in which he attempted to explain a disease known as "Jiggers," occurring in Alberta, Canada, as the result of anaphylactic poisoning due to sensitization towards *Gastrophilus* larvae.

The latest work on the question of anaphylaxis in relation to parasites appeared in 1924, when Bryce, Kellaway and Williams published their study on Hydatid Antigen, etc. (31). They found

that hydatid protein is capable of acting as an anaphylactic antigen. In 4 out of 14 cases, they met with a peculiar type of response in which a first testing dose gave a large reaction, almost certainly partly anaphylactic, and then subsequent doses gave a series of smaller contractions.

The transference of passive anaphylaxis to guinea pigs by the sera of infested human patients occurred only irregularly.

Technique of Isolated Anaphylactic Uterus Reaction.

Virgin female guinea pigs of which the weight is somewhat under 250 grams are best used, as the weight becomes 250 grams or a little over at the time at which they are ready for use. Inoculations were given subcutaneously in saline solution; in rare cases intraperitoneally. For passive anaphylaxis, anaphylactic or immune serum was given intraperitoneally.

When the guinea pig is to be used, it is stunned by a blow on the head, the throat is cut and the animal is allowed to bleed to death. If perfusion is to be performed, it is then laid out on a board and perfused; if not, the uterus is removed direct.

Perfusion.—The abdomen is opened up with scissors and the intestines lifted out of the cavity as far as possible. The mesorectum is then divided with sharp scissors and the rectum and accompanying blood vessels divided between double ligatures of cotton thread about 2 inches from the anus. The viscera are turned back, dividing the mesentery, and the body cut across above the level of the kidneys and the cranial half of the animal is rejected. We now have left the caudal half, with the kidneys and adrenals and bladder and uterus. A holding ligature is now put round the descending aorta below the exit of the renal arteries. Only the inferior mesenteric artery branches off now to viscera. The connective tissue is stripped back from the aorta for about an inch, taking care not to injure the vessels, and a loose ligature is passed round, but not tied yet. With points of a very sharp pair of fine scissors a small snip is made in the aorta about half an inch from the holding ligature, and the canula is inserted, using one as big as possible and with a T-piece to remove air bubbles. The canula is ligatured into position by tying the loose ligature. The flow of Ringer at 38°C. is started, and a snip made in the inferior vena cava to facilitate the exit of returning Ringer fluid. A frequently changed pad of warm (38°C.) Ringer is kept over the uterus to keep it warm and moist during perfusion. Perfusion is kept up till the uterus is blanched and bloodless, which, with a good stream issuing from the cut vena cava, takes about 15-30 minutes. The cervix and ovarian end become blanched first, the body last. When the uterus is quite bloodless, the canula is removed after closing the clip controlling the flow here.

Isolation of Uterus is now performed, and when perfusion is not carried out, the isolation is done straight after bleeding of the animal has ceased. Extreme care is used to avoid tension on the

uterus during removal, as this destroys its anaphylactic responding powers. The ovary is held with fine forceps, and no tension is exerted, and with sharp scissors the corpus uteri is bisected to the cervix and vagina. It is freed from the broad ligament, etc., and is cut away from the body and placed immediately in the spare bath of Ringer (at 38°C.) with oxygen bubbling.

Preparation for Experiment.—The other horn of the uterus is removed similarly and affixed to the hook which is suspended by a hair from the recording lever. The other (cervical) end is pushed on to the short platinum spikelet and thus fixed to the oxygenation tube. The preparation is submerged very gently into the Ringer bath, taking care not to stretch the muscle at all.

Ringer solution should be rejected if the temperature has been allowed to reach over 45°C., since partial decomposition of the sodium bicarbonate occurs, liberating carbon dioxide and the solution becomes alkaline. In any case, even the bubbling through of oxygen gradually displaces carbon dioxide, with a consequent rise in hydrogen-ion-concentration. Fortunately, the tolerance of the uterus to change in the hydrogen ion concentration is wide enough to prevent this latter factor from becoming of importance. When the uterus is immersed in the Ringer, it is in a state of contraction owing to the handling and the change of environment; and so it must be left till it gradually relaxes to its fullest extent, which takes from 15 to 30 minutes. The writing point is placed against the smoke-blackened drum, which is set in motion to see that the uterus has reached its maximum relaxation. Relaxation may be aided by the use of small weights which are hung on the arm of the lever, but great care must be exercised that excessive tension be avoided. A natural rhythm takes place after relaxation and the extent of this, which is quite small in a young (250 gram) guinea pig, should be noted and compared with the rise produced by addition of substances. The uterus remains suitable for use for 3-4 hours after removal from the body, but later the rhythm becomes exaggerated and erratic. Doorenbos (32), however, used in an experiment guinea pig uterus horns which had been kept in the ice chest overnight in Tyrode solution.

Method of Carrying Out the Test.—The maximum contraction of which the uterus is capable is determined either before the uterus is subjected to the action of any substance, or sometimes between the separate additions of various extracts, etc., or usually, after the substance under investigation has been added, and the effect of that substance has been observed.

For this purpose one used Beta-imidazolyl-ethylamine (otherwise Histamine), in the form of tablets of Ergamine acid phosphate (put up by Parke, Davis and Co.). Histamine is calculated as 1/3rd of the weight of the Ergamine acid phosphate. A concentration of 1 : 5,000,000 of the Ergamine acid phosphate causes a maximal contraction of the virgin guinea pig uterus (=0.01 mgm. in a 50 c.c. bath). By this means one can express the contraction given in response to the addition of any substance in terms of maximal response, e.g., a half-maximal response, etc.

Since many proteins are irritant to the isolated guinea pig uterus if in sufficient concentration (e.g., Horse Serum, *vide* Dale (3), Schultz (1), and in particular because many helminths are known to possess irritant properties, it is essential that any extract of helminth material under examination be first examined for its action on the normal virgin guinea pig uterus before it is applied to the sensitized uterus. For each extract and helminth preparation used, therefore, a series of preliminary tests had to be carried out to determine the maximum concentration of that extract that would not cause stimulation or other phenomenon such as loss of normal rhythm and diminished response to Histamine. This Minimum Non-Stimulating Concentration is called throughout the work, for the sake of brevity, M.N.-S.C., or, where the 50 c.c. bath is used throughout, the actual amount or dose of the extract itself is sometimes merely stated and called the M.N.-S.D.

Once the M.N.-S.C. is known, it is only necessary to set up an isolated uterus preparation from a suitable inoculated guinea pig (i.e., virgin and of reasonable weight) and to wait till the uterus is completely relaxed and is gently and regularly undergoing its normal rhythm. The Ergamine acid phosphate (known throughout the tests as simply "Histamine") may now be added to the 50 c.c. bath in amount of 0.03 mgm., and the maximal contraction recorded. When the writing point has reached its highest level the Kymograph is stopped and the Ringer fluid (at 38°C.) changed several times, to wash out all traces of Histamine, whereupon the uterus soon relaxes to normal and behaves as before. In order to see whether there is any sensitiveness to the protein under investigation, an amount corresponding to about 75% of its M.N.-S.C. is now added to the bath.

If now a contraction occurs, one can assume that it is due to sensitiveness to the extract, assuming the latter to be in the same condition as when it was tested for M.N.-S.C. This is, of course, always the case when freshly made up solutions of dry powdered helminths are used, but may not be so when stock saline extracts are used. These latter are very liable to undergo a decrease in the hydrogen-ion-concentration, on keeping, and furthermore must be kept under Toluol to prevent bacterial growth. Having procured a response to the addition of extracts, one allows the uterus to gradually relax to normal in the bath; this occurs fairly quickly with a 250-gram guinea pig in which the uterus is thin, but in bigger pigs relaxation may take a long while for completion. If the uterus is not allowed to relax in the bath, and if the bath is changed before this relaxation occurs, full desensitization may not have occurred, and one of the most striking and characteristic phenomena of anaphylactic shock, namely, desensitization, may not be observed. With these bigger uteri, especially if the bath is changed too soon, and before spontaneous relaxation has occurred, two additions of extract may be necessary before every smooth muscle cell is desensitized, and the uterus no longer responds to the normally sub-stimulating dose as it did previously.

After the anaphylactic contraction, if any, has been observed, the Histamine may now be added in order to obtain the maximal contraction.

Since parasites are usually in close association with the tissues of their hosts, and are thus liable to be contaminated with host proteins, it is necessary to consider this important factor when experimenting with them. Even with the intestinal worms, possible contamination with serum or perhaps bowel-wall proteins must be kept in mind, and during the test eliminated by the preliminary addition to the bath, before the parasite extract is added, of extracts containing these non-parasite proteins. In the case of an anaphylactic response to them, absolute desensitization to them must be obtained before the parasite extract is added. In the case of parasites, which are so intimately connected with the tissues of their host that absolute freedom from contamination is extremely difficult, if not impossible, this factor is inevitable. This is the case with extracts of *Onchocerca,* where Ox serum is a contaminant.

It is also a fact, as shown in this work, that a uterus sensitized to a particular nematode or arthropod protein may show sensitiveness to protein of parasites belonging to the same genus, or even order. Hence, if one were endeavouring to prove the identity of two helminths or arthropods by the anaphylactic response to their respective extracts, one could very easily be led astray, and by apparently specific anaphylactic responses, with subsequent desensitization, one might assume the identity of helminths which were perhaps generically distinct. Hence in these cases, this disturbing, though interesting fact, must be allowed for, and may be eliminated as far as the present experiments show for the nematodes used, by the addition of some known nematode extract not identical with the one used for sensitizing. This phenomenon of non-specific sensitization was first observed by Dale when dealing with crystallized egg albumins of the duck and the fowl, though here the two proteins are much further zoologically removed than the proteins of the two arthropod larvae or nematodes used.

Apparatus.—The apparatus used is essentially that described by Burn and Dale (33) in their work on the Standardization of Pituitary Extracts. A short description will be given for the advantage of those who cannot refer to the original article.

It consists of a thermostat bath made of copper, cylindrical in shape. The flat bottomed bath stands on the table, which also supports the recording drum. A hole in the table allows the passage of the glass tube leading to and from the testing vessel, which consists of a cylindrical glass vessel about 2.5 cm. in diameter, and which, when filled to a certain mark, holds 50 c.c. Originally we used baths of 100 c.c. capacity, but found that it was quite practicable to reduce the cubic content of the vessel, thus bringing about a big saving in the consumption of our protein extracts, some of which we had only in limited amounts.

The testing vessel is produced into a glass tube of $\frac{1}{4}$ inch diameter, which passes through a rubber stopper in the centre of the bottom of the bath and joins a T-tube, one arm of which is continued as a rubber tube, with a pinch-cock, leading to a waste-bucket. The other arm joins a rubber tube, also with a pinch-cock near the junction, and this rubber tube is the inferior extremity of a syphon system leading from a large glass beaker of 3 litre capacity, which is supported on an iron tripod over a bunsen burner about 2′6″ above the table, on a high stool. Once the syphon system is established, filling and emptying of the testing bath is accomplished by a simple manipulation of the pinchcocks. The water bath is kept at 38°C., after being filled by water at that temperature, by means of a carbon filament lamp which can slide to a variable distance into a recess tunnel built into the side of the bath at the bottom. The lamp is adjusted to varying degrees of penetration into the tunnel, depending on the room temperature, and the bath is thus easily kept at the required temperature. The storage vessel, which holds Ringer solution, is kept at 38°C. by means of the adjustable pilot flame of the burner and gives quite a satisfactory constant temperature to the Ringer.

The uterus is fixed in the testing bath by means of a bent glass tube (of $\frac{1}{4}$″ diameter), the lower end of which is sealed off, and in which is inserted a short piece of stout platinum wire. About an inch from the end is blown a tiny hole in the side of the tube, through which oxygen passes, and bubbles up through the Ringer solution. The contractions of the muscles are recorded by a light, easy-moving lever, from one end of which hangs a hair which is fastened to a bent entomological pin. The ovary is fastened to the hair by the bent pin, and the cervical end of the uterine horn is impaled on the platinum spike, which is a fixed point.

The lever itself consists of a strip of light thin steel soldered to a half of the balance wheel of a clock. This is supported in a frame by means of the centred screws belonging to the balance wheel. The other end of the lever has attached to it a light straw extension, in which lies a piece of thin glass tubing, so bent that its tip can be adjusted at right angles to the surface of the Kymograph, which is covered with smoked glazed paper. The arrangement of the bent glass tubing ensures that the writing point follows the surface of the drum during all excursions of the lever. The drum is turned very slowly by an electric motor.

In our earlier experiments, the uterus was perfused with Ringer solution before being tested, but this procedure was soon discarded, as it appeared to offer no advantages over simply setting up the muscle as taken directly from the animal. For purposes of perfusion we used a glass canula, consisting of a glass bulb about $1\frac{1}{2}$″ long by $\frac{3}{4}$″ in diameter, in one end of which entered a piece of glass tubing of $\frac{1}{4}$″ diameter. At the other end of the bulb was a fine constricted tube, which was tied into the aorta. At one side of the bulb was a short piece of tubing with rubber tubing and pinch cock, the purpose of which was to pro-

vide an exit for any air bubbles which may have reached the canula. The Ringer came from the same syphon system described above by means of a side tube and stop-cock.

Ringer Solution.—This must be made up from pure chemicals and glass distilled water, i.e., water which has been condensed and received on a glass surface, such as a Liebig condenser.

Stock Solution 1.

Sodium chloride	450.0 grams.
Potassium chloride	21.0 grams.
Sodium bicarbonate	25.0 grams.
Glass-distilled water	2500.0 c.c.

Dissolve the two chlorides in a sufficient amount of the water using warmth if necessary; and dissolve the bicarbonate in more water (cold or below 45°C.). Mix the two and make up to 2½ litres.

Stock Solution 2.

Calcium chloride (anhydrous)	12.0 grams.
Glass-distilled water	100.0 c.c.

Preparation.—Take 50.0 c.c. of Solution 1, and add to it about 800 c.c. of glass distilled water. Add 1.0 c.c. of Solution 2, and make up the whole to 1 litre. Then add 1.0 gram of pure glucose. It should be made up as required daily, but may be kept overnight if needed next morning.

Normal Saline Solution.—Throughout this work, this means a 0.85% solution of pure Sodium Chloride (analytical reagent) in glass distilled water.

Preliminary experiments with common antigens.

G.P. O/40. Received 24 days before 0.1 c.c. of Ox Serum subcutaneously. Bath 50 c.c. capacity.

Horn A. Added 0.01 c.c. Ox Serum (1: 5,000) —Rapid maximal contraction.
Changed Ringer.
Added 0.01 c.c. Ox Serum —Very slow rise to ½ maximal.
Allowed to relax.
Added 0.01 c.c. Ox Serum —No response, hence desensitized.
Added 0.0005 gr. Histamine —Maximal contraction.
Horn B. Not tested.

This experiment did not attempt to exploit the delicacy of the reaction, as a big concentration (1 : 5,000) was used.

G.P. O/57. Received 35 days before 0.1 c.c. of Rabbit Serum.

Horn A. Added 0.05 c.c. Rabbit Serum (1: 1,000) —Maximal contraction.
Allowed to relax.
Added 0.05 c.c. Rabbit Serum —Very slight rise of ½ inch, slowly coming to normal.
Added 0.05 c.c. Rabbit Serum —No rise, hence fully desensitized.
Horn B. Exactly the same results as with Horn A.

The following experiment had the double object of determining the effect of moderate concentrations of Toluol on the sensitive uterus and of seeing whether the delicacy of the reaction was impaired by the presence of Toluol.

G.P. O/19. Wt. 430 grams. Received 74 days before 0.1 c.c. Horse Serum subcutaneously. Bath=50 c.c.

Added 1 c.c. of Saturated Solution of Toluol in Normal Saline.	—No rise.
Added 0.00005 c.c. Horse Serum (1: 1 million)	—Maximal contraction.

Illustrating the specificity of the reaction.

G.P. O/111. Wt. 250 grams. Received 12 days before 1.0 c.c. of Amniotic Fluid of Rabbit.

Horn A. Added 0.1 c.c Rabbit Serum	—Maximal rise.
Allowed to relax.	
Added 0.1 c.c. Rabbit Serum	—No effect. Desensitized to Rabbit Serum.
Changed Ringer.	
Added 0.1 c.c. Amniotic fluid	—No effect.
Horn B. Added 0.05 c.c. (1: 1,000) Horse Serum	—No rise.
Added 0.05 c.c. (1: 1,000) Ox Serum	—No rise.
Added 0.05 c.c. (1: 1,000) Sheep Serum	—No rise.
Added 0.00005 c.c. (1: 1,000,000) Rabbit Serum	—Halting rise to nearly half maximal, returning to normal.
Added 0.01 c.c. (1: 500) Rabbit Serum	—Maximal rise.
Relaxed.	
Added 0.01 c.c. (1: 500) Rabbit Serum	—No rise, hence desensitized.

The result with Horn A is also interpreted as a proof that amniotic fluid does not contain proteins differing from those in blood-serum.

The following was a repetition of one of Dale's experiments on the specificity of the reaction even with two such closely related proteins as hen egg albumin and duck egg albumin, excepting that here the ordinary egg white was used while Dale worked with the purified albumins. Fresh eggs were used and dilutions of the white were made before the test and used.

G.P. O/21 Wt. 435 grams (note the large weight). Received 62 days before 0.1 c.c. of Hen Egg-White subcutaneously. Bath=50 c.c. capacity.

Horn A. Added 0.1 c.c. (1: 500) Ox Serum	—Nil.
Ringer changed.	
Added 0.1 c.c. (1.500) Horse Serum	—Nil.
Ringer changed.	
Added 0.005 c.c. (1: 10,000) Duck Egg-White.	—Maximal contraction.

Added 0.1 c.c. Duck Egg-White (1: 500) —Almost maximal contraction.
Added 0.1 c.c. Duck Egg-White—A ¾ maximal contraction, but extremely drawn-out rise.
Added 0.1 c.c. Duck Egg-White —No response, hence desensitized to Duck Egg-White.

Ringer changed.
Added 0.000005 c.c. (1: 10 millions) Hen Egg-White. —No rise within a minute.
Added 0.00005 c.c. (1: 1 million) Hen Egg-White. —A maximal contraction.

This experiment of Dale's illustrates the fact that while often there is considerable sensitiveness to the non-specific allied albumin, yet on desensitizing to this we can still get the specific sensitiveness to the egg albumin used for sensitization.

Commentary on the above Experiments.—These experiments illustrate that the anaphylactic reaction can occur in a concentration of the Antigen of 1 : 1,000,000 of Ringer; that Toluol does not affect this degree of sensitiveness; that the reaction is very specific; and that sometimes in proteins from very closely related sources sensitiveness to one protein may be accompanied by sensitiveness to the allied protein, but that this non-specific sensitiveness may be exhausted and yet the specific sensitiveness will be still obtained.

Effect of some chemicals on the isolated guinea pig uterus.

Phenol.—Carbolised antigens of Onchocerca material were first used on account of the ease with which they keep free from bacterial contamination, but it was realised after much work at Glenfield, N.S.W., that the phenol was harmful to the uterus, and that it probably in large part accounted for the negative results obtained there.

These carbolised antigens, however, are quite good for sensitizing purposes.

The effect of Phenol, in the form of Carbolised Saline Solution, is shown in the following experiment. The carbol saline in use at this laboratory has the composition—

Phenol	0.5
Sodium Chloride	0.85
Water	100.0

Normal G.P. O/49. Bath=50 c.c. capacity.
Set up and allowed to relax.
Added 0.01 c.c. of Carbol Saline—A rise of nearly an inch after about 2 minutes. Uterus gradually returned to normal.

Changed Ringer.
Added 0.0025 c.c. Carbol Saline —No effect.

From this experiment it seemed that it was not advisable to use even 0.01 c.c. of a carbolised antigen=a concentration of

1 : 100,000 Phenol. Hence it was seen that it was useless to employ such extracts for testing. It was not considered necessary to try the effect of Phenol on the anaphylactic responses of a sensitized uterus.

Chloroform.—This substance was at one stage of the work considered as a possible preservative agent to combine with normal saline solution in the form of a saturated solution, to be used for forwarding Nematode-infested Tabanids to Melbourne.

The result of the following experiments, taken together with an oral communication from Professor Osborne, of the Physiology School, University of Melbourne, that chloroform is able under certain conditions to precipitate at least some albumins, certainly lactalbumin, resulted in chloroform being discarded. Chloroform was used in the form of Aqua chloroformi, B.P., which represents a 0.25% aqueous solution.

Normal G.P. O/49. Bath 50 c.c. capacity.

Set up and allowed to relax.

Added 0.1 c.c. Aqua Chloroformi (B.P.) —No effect.

Added 0.5 c.c. Aqua Chloroformi (B.P.) —A sudden relaxation of the muscle denoted by a fall of the pointer and decreased amplitude of the rhythm.

Changed Ringer.

Added 0.25 c.c. Aqua Chloroformi —No effect.

Changed Ringer.

Added 0.5 c.c. Aqua Chloroformi—Sudden relaxation resulting in a drop of pointer of ¼ in. Amplitude little affected.

Amplitude of rhythm gradually increased till it was almost twice that which was possessed by the uterus at the beginning of the experiment. Frequency also slightly diminished. This phenomenon may have been similar to the usual exaggerated rhythm seen after a uterus has been suspended in the Ringer for some time.

Added 1.0 c.c. Aqua Chloroformi—No further effect.

From this experiment it was seen that 0.5 c.c. of Aqua Chloroformi (B.P.)—which contains 0.25% of Chloroform in water—was inimicable to the normal uterus. This equals a concentration of 1 : 40,000 of Chloroform.

Toluol.—This substance was chosen as the preservative and bacteriostatic agent needed. It is also used to cover any fluid antigens we possess. The experiment with it described on page 36 is set out again for the sake of continuity.

This experiment had the double object of determining the effect of moderate concentrations of Toluol on the sensitive uterus and of seeing whether the delicacy of the reaction was impaired by the presence of Toluol.

G.P. O/19. Wt. 430 grams. Received 74 days before 0.1 c.c. Horse Serum subcutaneously. Bath 50 c.c.

Added 1 c.c. of Saturated Solution of Toluol in Normal Saline	—No rise.
Added 0.00005 c.c. Horse Serum (1: 1 million)	—Maximal contraction.

Glycerine.—Later on in the course of the experiments, it was found that Toluol possessed some grave disadvantages for the purpose for which it was used in the transporting of material. On the advice of Associate-Professor Young, glycerine was investigated and found to be without effect on the normal uterus in concentrations far above those to which it would be subjected in the course of the testing of material forwarded in a 5% normal saline solution of glycerine.

Normal G.P. N/13. Wt. 240 grams. Bath 50 c.c. Set up and allowed to relax with a normal rhythm, using a 5% Normal Saline Solution of Glycerine.

Added 0.5 c.c. (=1: 2,000 concentration of Glyc.)	—No effect.
Added 1.0 c.c. (=1: 1,000 concentration of Glyc.)	—No effect.
Added 2.0 c.c. (=1: 500 concentration of Glyc.)	—No effect.
Added 5.0 c.c. (=1: 200 concentration of Glyc.)	—No effect.
Added 0.05 mgm. Histamine	—A max. contraction, though a little slower than usual.

Hence the total amount of Glycerine solution added was 8.5 c.c. =a glycerine concentration of about 1 : 138, and the uterus was subjected without apparent irritation, to 1 : 200 concentration at one dose, or a cumulative concentration of 1 : 138.

Sensitising Powers of Various Parasites.

A. Sensitizing Powers of Ascaris equi.

(a) *Preparation of Extract*—

Adult *Ascaris equi* were collected from the intestine of a horse at post mortem. After washing several times in Normal Saline, they were dried with filter paper and cut up in a sterile Petrie dish and placed in the incubator at 37°C. to dry. After 5 days they were quite dry and powdered readily in a mortar.

For use, the powder was shaken with normal saline solution (made with pure Sodium chloride and glass distilled water) in the proportion of 0.1 gram of powdered worm to 10.0 c.c. of saline. Extraction was then occasionally aided in some cases by a few hours in the incubator at 37°C., with shaking at intervals; but usually the mixture was placed in the ice-chest overnight. A small amount of Toluol was added to inhibit bacterial growth.

Before use the mixture was shaken again, and the extracted powder removed by centrifugalisation. The use of normal saline solution ensured that both the globulins and the albumins would be extracted, assuming both or either to be present.

(b) *Determination of M.N.-S.C.*—

This was decided on as 1 : 2,500, and the largest dose that was used in the 50 c.c. bath was 0.01 gram (=a concentration of 1:5,000.

(c) *Test Experiments*—

G.P. O/52. Wt. 295 grams. Bath 50 c.c. capacity. Inoculated 36 days before with the normal saline soluble proteins in 1.0 mgm. of dried powdered Ascaris equi.

Horn A. Set up and allowed to relax.
Added 0.01 c.c. Horse Serum —No effect.
Changed Ringer.
Added 0.25 c.c. Antigen I. —Sudden maximal contraction.
(Ringer extract of Worm Nodules)
Changed Ringer.
Added 0.25 c.c. Antigen I. —Halting rise to ¾ maximal.
Changed Ringer.
Added 0.1 c.c. Antigen I. —No effect.
Changed Ringer.
Added 0.0001 gram dried Ascaris equi (1: 500,000) —Very slight rise of ½ inch.
Added 0.001 gram (1:50,000) of Ascaris —Halting maximal contraction.
Horn B. Added 0.01 gram Ascaris equi —Sudden maximal contraction.
Allowed to relax.
Added 0.01 gram Ascaris equi —No effect.
Hence desensitization to the specific Antigen.
Changed Ringer.
Added 0.1 c.c. Onchocerca extract (Antigen I.) —No effect.

G.P. O/53. Wt. 400 grams. Received 90 days before 1.0 mgm. dried Ascaris equi.

Set up and allowed to relax. (Owing to extreme length of uterus only about one half of the horn was used.)
11.27 a.m. Added 0.25 c.c. Antigen I. —No rise.
(Ringer Extract of Worm Nodules)
11.33½ Added 1.0 mgm. Toxascaris limbata —No rise.
11.36 Added 5.0 mgm. T. limbata —No rise.
11.38 Added 1.0 mgm. Ascaris equi—Maximal contraction.
Allowed to relax. Stopped motor.
12.11 p.m. Added 1.0 mgm. Ascaris equi—No rise.
Hence desensitized to A. equi.
Added 0.1 mgm. Histamine —Maximal contraction.

Discussion.—In this uterus, there was sensitiveness to only the specific protein of Ascaris. No sensitiveness to the allied worm Toxascaris.

G.P. 137. Wt. 268 grams. Received 21 days before 2.5 mgm. A. equi. Horn A. Set up.

Relaxed.	
Added 1.0 mgm. A. equi	—Quick maximal rise.
Changed Ringer.	
Added 10.0 mgm. A. equi	—Rise to maximal.
Changed Ringer.	
Relaxed.	
Added 10.0 mgm. A. equi	—No effect.
Therefore desensitized.	
Added 10.0 mgm. A. suilla	—No effect.
Changed Ringer.	
Added 10.0 mgm. T. limbata	—No effect.
Added 0.05 mgm. Histamine	—Rise to maximal.

B. Sensitizing Powers of Toxascaris limbata.

(a) *Preparation of Extract.*—The worms were dried and powdered as for Ascaris and 1% extracts of their protein made.

(b) *Determination of M.N.-S.C.*—1 : 5,000 is the concentration to be used.

(c) *Experiments.*—

G.P. O/60. Wt. 315 grams. Bath=50 c.c. capacity. Received, 50 days before, the normal saline-soluble proteins in 1 mgm. of dried, powdered Toxascaris limbata.

The effect of serums of Horse and Ox were tried, as these may have been present in the small intestine (as food constituents) with the Ascarids.

Horn A. Set up and allowed to relax.

Added 0.1 c.c. Horse Serum	—Gradual rise to 1 inch and gradual fall to normal.
Added 0.1 c.c. Horse Serum	—A very slight rise (¼ in.)
Added 0.1 c.c. Horse Serum	—No effect.
Added 0.1 c.c, Ox Serum	—No effect.
Changed Ringer.	
Added 0.2 c.c. Antigen I. (Ringer extract of worm nodules)	—Almost maximal contraction.
Allowed to relax and changed Ringer.	
Added 0.2 c.c. Antigen I.	—Very slight rise (¼ in.).
Changed Ringer.	
Added 0.2 c.c. Antigen I.	—No effect.
Hence desensitized to Onchocerca.	
Added 0.25 c.c. Strongylus equinum extract	—No effect.
Added 1 mgm. Ascaris equi	—No effect.
Added 5 mgm. Ascaris equi	—No effect.
Added 1 mgm. Toxascaris limbata	—Sudden maximal contraction, which showed a tendency to take a long time to relax.
Ringer changed.	
Added 1.0 mgm. Toxascaris	—No effect.
Added 1.0 mgm. Toxascaris	—No effect.
Hence desensitized to Toxascaris.	
Added 0.1 mgm. Histamine	—Maximal rise.

Horn B. Added 0.1 c.c. Horse Serum —No effect.
Added 0.1 c.c. Ox Serum —No effect.
Added 2.5 mgm. Ascaris equi —Rise to 1 inch.
Allowed to relax.
Added 2.5 mgm. Ascaris —No effect.
Hence desensitized to Ascaris equi.
Changed Ringer.
Added 0.2 c.c. Onchocerca Nodule extract Antigen 1 —No rise.
Added 0.2 c.c. Antigen 1 —No further effect.
Added 0.005 mgm. Toxascaris limbata —No effect.
Added 0.025 mgm. Toxascaris —No effect.
Added 0.075 mgm. Toxascaris —No effect.
Added 1.0 mgm. Toxascaris —Maximal contraction.
Allowed to relax.
Added 1.0 mgm. Toxascaris —No effect.
Hence desensitized to Toxascaris limbata.
Added 0.1 mgm. Histamine —Maximal rise.

C. Sensitizing Powers of Ascaris suilla.

(a) *Preparation of Extract.*—A large number of specimens of Ascaris suilla was collected from the intestines of pigs at the Abattoirs. After being washed in saline, they were cut up into small pieces in a Petrie Dish, and dried in vacuo over concentrated sulphuric acid. When dried, the mass was powdered and stored in the ice chest. For use a 1% extract was made in normal saline and left overnight in the ice chest. Here again, Toluol was placed over the extract for preservation.

(b) *Determination of M.N.-S.C.*

Normal G.P. N/11. Wt.=290 grams.

Horn A.
2.0 p.m. Set up.
2.20 Relaxed.
2.21 Added 10.0 mgm. A. suilla —No effect.
2.22 Added 20.0 mgm. A. suilla —Slight rise of ⅛ in.
(Total 30.0 mgm.)
2.25 Added 0.05 mgm. Histamine —Rise to maximal
Hence from the above, Minimum Stimulating Dose is between 10.0 and 30.0 mgm.

Horn B.
2.33 p.m. Set up.
3.15 Relaxed.
3.17 Added 20.0 mgm. A. suilla —Slight rise of ⅛ in.
Changed Ringer.
3.25 Relaxed.
3.26 Added 10.0 mgm. A. suilla —No rise.
5.27 Added 5.0 mgm. A. suilla
(Total 15.0 mgm.) —No rise.
3.28 Added 5.0 mgm. A. suilla —Merely a very slight transient
(Total 20.0 mgm.) rise.
3.29½ Added 5.0 mgm. A. suilla —Slight rise, returning to nor-
(Total 25.0 mgm.) mal slowly.
3.33½ Added 5.0 mgm. A. suilla
(Total 30.0 mgm.) —No response.
3.35½ Added 5.0 mgm. A. suilla
(Total 35.0 mgm.) —No response.

3.37	Added 5.0 mgm. A. suilla (Total 40.0 mgm.)	—Larger amplitude.
3.40	Added 5.0 mgm. A. suilla (Total 45.0 mgm.)	—Slight rise, and then a rise of ½ in. at 3.42 slowly relaxing.
3.57½	Added 5.0 mgm. A. suilla (Total 50.0 mgm.)	—No rise.
4.0	Added 5.0 mgm. A. suilla (Total 55.0 mgm.)	—No rise.
4.2.	Added 5.0 mgm. A. suilla (Total 60.0 mgm.)	—Rise at 4.3½ p.m. of about 1 in., relaxing again.
4.5	Added 0.05 mgm. Histamine	—Rise to maximal.

This experiment shows the tolerance which the uterus can acquire towards an irritant substance when it is added in gradual doses.

In this case a rise of only 1″ was obtained when the bath held 60.0 mgm. of A. suilla, which dose would certainly be stimulating to the uterus if added at once.

From the above the Minimum Stimulating Dose lies between 10 and 20.0 mgm., the latter giving only a very slight rise of ⅛″. The Minimum N.-S.D. may thus be taken as 15 mgm., and 10·0 mgm. may be added to a testing bath with safety.

(c) *Test experiments*—

G.P. O/117. Wt. 385 grams. Received 44 days before 5.0 mgm. A. suilla.

Horn A.

2.32 p.m.	Added 5.0. mgm. A. equi	—After a pause of half a minute, there was a stepping rise to maximal which relaxed to normal by 2.37.
2.40	Added 5.0 mgm. A. equi	—After a pause of 1½ minutes, a stepping rise to ½ maximal, relaxing by 3.47.
	Changed Ringer.	
2.58	Added 5.0 mgm. A. equi	—No rise.
	Therefore desensitized to A. equi.	
3.0	Added 5.0 mgm. T. limbata	—No rise.
3.2	Added 1.0 mgm. A. suilla	—No rise.
3.4	Added 5.0 mgm. A. suilla	—After a pause of 2 minutes there was a stepping rise to ¾ maximal, relaxing by 3.17 p.m.
3.18	Added 5.0 mgm. A. suilla	—No rise.
3.22	Added 0.01 mgm. Histamine	—Sudden maximal rise.

Horn B.

3.48 p.m.	Added 1.0 mgm. A. suilla	—After pause of ¾ minute there was a maximal contraction which relaxed.
4.7	Added 10.0 mgm. A. suilla	—Stepping rise to ½ maximal, relaxed by 4.12 p.m.
4.13	Added 5.0 mgm. A. suilla	—No rise.
	Therefore desensitized to A. suilla.	
4.15	Added 5.0 mgm. A. equi	—No rise.
4.19	Added 5.0 mgm. T. limbata	—No rise.
4.44	Added 0.05 mgm. Histamine	—Maximal contraction.

D. Sensitizing Powers of Onchocerca gibsoni.

(a) *Preparation of Onchocerca Extracts.*—The following extracts were prepared:—

A. Carbolized Saline Extract of Worm Nodule.—Onchocerca nodules were bisected and the fibrous reticulum and pieces of worm scraped out and placed in carbolized saline solution and ground with powdered glass to a fine mulch. The mixture was put in the incubator for a week, at 37°C. Though this extract proved later to have sensitizing properties, it was decided to discard it for two reasons—firstly, that being carbolised it was not fit for actual testing of the uteri on account of the high phenol content (0.5%), and secondly, because it was later realised that incubation of extracts for prolonged periods was bad in principle, because of the liability to lysis and formation of breakdown products.

B. Four nodules were incised, and the pieces of adult female worm removed and placed in normal saline, and shaken in a shaker for half an hour in the hope of removing most of the larvae and ova from the worm. The pieces of female were then picked out and washed eight times by repeated hand shaking in saline, and then centrifuging. They were then ground up by the aid of glass in 40 c.c. of carbolized saline, and placed at 37°C. for three days.

C. A similar extract was made with the exception that the final grinding up was done in normal saline solution. The ground up material and fluid were placed in a test tube, and hermetically sealed and sterilized by exposure in a water bath at 55°C. for one hour on three consecutive days.

D. The saline in which dissection of the nodules had been carried out in preparation of antigens B. and C. was collected after the pieces of adult and many of the embryos and ova had been thrown down in the centrifuge. It was divided into two portions. One portion was filtered to remove debris and embryos and ova, and sealed in a test tube and sterilized fractionally. It was thought that this fluid, presumably containing coelomic fluid of the adult female, as well as serum of the host and perhaps some constituent protein of the fibrous tissue of the nodule, might serve as a sensitizing antigen.

E. The other portion was boiled, the reaction of the fluid being neutral, and the coagulum as well as ova and embryos and debris was filtered off. The filtrate was sealed in a tube, and also passed through the fractional sterilizing with the other non-carbolized antigenic fluids. It was thought that possibly the coelomic fluid might contain some protein not coagulated by heat, similar to caseinogen of milk, but which could act as an antigen. In the event of such being the case, the double object would have been served by ridding the fluid of host proteins (albumins and globulins), and thus of presenting a purer antigen with consequent increase in the delicacy of the reaction. These hopes, as seen later, were not realised.

J. Twelve nodules were dissected and the pieces of adult taken out and ground in 50 c.c. of normal saline solution. Filtered through Gooch crucible and stored under Toluol.

(b) *Precautions and desensitizing agents against non-verminous proteins.*—Since *Onchocerca gibsoni* is a parasite of the subcutaneous and intermuscular connective tissue of the Ox, it is evident that extracts of Nodules will contain proteins of the host—such as blood serum proteins and perhaps proteins peculiar to connective tissue cells. Hence in experiments in which guinea pigs were sensitized to extracts of worm nodule, the necessity arises to desensitize the uterus to ox serum. The possibility that sensitiveness to fibrous tissue cell protein might exist, must not be neglected, and so an extract of fibrous tissue was prepared by grinding in normal saline with glass some aponeurosis and tendon of an ox. This extract, however, appeared to have no effect on the uterus sensitized, and so probably no sensitiveness to fibrous tissue cell protein exists.

As Dale has shown, the purer the Antigen used for sensitization, the more delicate the degree of sensitiveness, and also with multisensitization the degree of sensitiveness to any of the antigens used is much less than it would have been if the uterus had been sensitized to that protein only, and also desensitization to one antigen is not without effect on the degree of sensitization towards the others.

For these reasons it was thought desirable to reduce the number of non-specific proteins in the extracts to as few as possible, and hence an extract of the dissected-out adult female worm was used. This extract gave good results. Guinea pigs sensitized with this extract showed sensitiveness towards ox serum in two cases and not in another.

(c) *Tests performed to determine whether Onchocerca protein could sensitize the uterus.*—The principle in these tests was to inoculate a guinea pig subcutaneously with an arbitrarily determined amount of antigen, in this case 0.1 c.c. After an interval of three weeks or more they were tested in the following manner. After setting up in the Ringer bath and allowing to relax, one added the non-verminous proteins which one might consider to be possibly present in the extract. The two which come to mind are normal ox serum and some extract of fibrous tissue. If any reaction occurred on the addition of these substances a further dose was given, till the uterus was desensitized to these proteins. Then the M.N.-S.D. of the antigen under consideration was given—if then an anaphylactic contraction occurred it was assumed to be due to anaphylactic shock brought about by sensitization towards the verminous protein.

G.P. O/25. Received 61 days before 0.1 c.c. of Antigen A.
Antigen A = carbolized saline extract of worm nodule.
Antigen E = dissection fluid (boiled).
Antigen D = dissection fluid (not boiled).

Set up and allowed to relax —This uterus was rather oestral.
Added 0.05 c.c. Ox Serum —Maximal contraction.
Allowed to relax to normal.
Added 0.05 c.c. Ox Serum —No typical rise, but a gradual contraction of the uterus.
Changed Ringer.
Added 0.25 c.c. Ox Serum —No effect.
(To be sure of the ox serum desensitization)
Changed Ringer.
Added 1.0 c.c. Fibrous tissue extract —A slight transient rise of ½ inch.
Added 1.0 c.c. Fibrous tissue extract —Another rise, but uterus began to become erratic.
Ringer changed.
There appeared to be no special sensitiveness to Fibrous tissue protein.
Added 0.025 c.c. Antigen C. —A maximal contraction.
Changed Ringer.
Added 0.025 c.c. Antigen C. —No effect.
Hence desensitized to the adult Onchocerca.
Added 0.12 c.c. Antigen F. (All that remained) —No effect.
Changed Ringer.
Added 1.0 c.c. Antigen E. —No effect.
Added 0.25 c.c. Antigen D. —No effect.
Added 0.001 mgm. Histamine —Maximal contraction.

The only reliable result from this experiment was the definite sensitization to adult Onchocerca, and to Ox Serum, as we would expect.

G.P. O/31. Sensitized 53 days before with 0.1 c.c. of Antigen C., prepared by triturating adult female *Onchocerca gibsoni* in Normal Saline Solution. The M.N.-S.D. of this antigen was found to be 0.025 c.c. or a M.N.-S.C. of 1: 2,000.

Set up and allowed to relax —Uterus is somewhat oestral.
Added 0.5 c.c. of Normal Saline Extract of Ox Fibrous Tissue (Necessarily containing Ox Serum) —A maximal contraction, taking a few steps to attain maximum height.
Changed Ringer.
Uterus showed very erratic rhythm and a tendency to gradually contract.
Added 0.001 mgm. Histamine —Maximal contraction.
Changed Ringer.
Added 0.025 c.c. Antigen C. —Sudden maximal contraction.
Changed Ringer.
Added 0.025 c.c. Antigen C. —Sudden almost maximal contraction.
Changed Ringer.
Added 0.025 c.c. Antigen C. —A very halting and oscillating rise to almost maximal.
Changed Ringer.
Added 0.025 c.c. Antigen C. —No effect.
Hence desensitized here.
Added 0.01 mgm. Histamine —Maximal contraction.

In this experiment, although one should have made sure of the desensitization towards host proteins, yet the intensity of the

reactions following addition of Antigen suggests that the uterus was sensitive towards this Antigen.

Discussions.—These experiments on *Onchocerca* protein show that this protein is an anaphylactogen, giving a good reaction with the Antigen after removing the sensitiveness due to host proteins (serum). This result has been obtained on four other occasions.

The case of G.P.O/25 should be compared with the findings of Bryce, Kellaway and Williams (34), who noted, in 4 cases out of 14, using as antigen hydatid (*Echinoccus*) fluid, a peculiar type of response, in which a large reaction is obtained with the first dose of antigen, but this does not exhaust the muscle which goes on reacting repeatedly to subsequent doses of hydatid fluid, the Ringer in the bath being changed after each response. The large primary reaction in our case must surely be conceded as at least partly anaphylactic in origin; and the fact that the animal was a big one of weight, about 400 grams, might suggest that the second shock was due to incomplete desensitization of a big uterus horn. But the occurrence of a third though less intense shock seems to be to other causes than anaphylaxis. Note that the uterus was entirely insensitive to the antigen after this third dose. This phenomenon was not noticed again, so that it occurred with one out of five animals tested.

E. Sensitizing Powers of larvae of Gastrophilus haemorrhoidalis.

(a) *Preparation of Extract.*—*Gastrophilus haemorrhoidalis* larvae were collected from the stomach of a horse at post mortem, and after washing and identifying, were cut up and dried as for Ascaris, and then powdered. 100 milligrams extracted with 10 c.c. normal saline (1%).

(b) *Determination of M.N.-S.C.*

(c) *Experiment.*

G.P. O/50. Wt. 370 grams. Received 49 days before the Saline soluble proteins in 1.0 mgm. G. haemorrhoidalis larvae (dried and powdered). As possible host proteins, horse serum and a 25% extract in normal saline of horse's stomach were used. Set up and allowed to relax.

Added 0.1 c.c. Horse Serum	—No effect.
Added 0.1 c.c. Extract of Horse's stomach	—No effect.
Added 1.0 mgm. G. nasalis	—A slight rise of 1 inch, relaxing to normal.
Added 0.5 c.c. Extract of Stomach	—No effect.
Changed Ringer.	
Added 0.05 mgm. Histamine (As uterus had lost its rhythm)	—Maximal contraction of 4½ inches.
Changed Ringer.	
Added 5.0 mgm. G. nasalis	—Immediate rise of 3 inches.
Allowed to relax.	
Added 5.0 mgm. G. nasalis	—No effect.

Added 5.0 mgm. G. nasalis —No effect.
Hence desensitized to G. nasalis.
Added 1 mgm. G. haemorrhoidalis —Immediate rise of 3 inches.
Allowed to relax.
Added 1 mgm. G. haemorrhoidalis —No effect.
Added 2.5 mgm. G. haemorrhoidalis —No effect.
Hence desensitized to G. haemorrhoidalis.
Added 0.5 mgm. Histamine —Rise of 3 inches.

Horn B. Added 0.1 c.c. Horse Serum —No effect.
Added 0.5 c.c. Extract of Stomach —No effect.
Added 1.0 mgm. Ascaris equi —No effect.
Added 10.0 mgm. Ascaris equi —No effect.
Added 1.0 mgm. G. haemorrhoidalis. —Maximal contraction.
Allowed to relax.
Added 1.0 mgm. G. haemorrhoidalis —No effect.
Added 1.0 mgm. G. haemorrhoidalis —No effect.
Hence desensitized to G. haemorrhoidalis.
Added 5.0 mgm. G. nasalis —No effect.
Added 0.05 mgm. Histamine —Maximal contraction.

G.P. O/51. Wt. 410 grams. Received 86 days before 2.0 mgm. dried G. haemorrhoidalis.

Set up and allowed to relax.
Added 0.5 mgm. G. nasalis —No response.
Added 0.05 c.c. Horse Serum —No rise.
Added 5.0 mgm. G. nasalis —No response.
Added 0.5 mgm. G. haemorrhoidalis (=1: 100,000 of dried larvae) —Rise to maximal.
Stopped motor and allowed horn to relax in the exciting medium to procure desensitization.
Added 5.0 mgm. G. haemorrhoidalis —After a long interval there was a halting and oscillating rise to nearly half maximal coming down soon in a typical fashion.
Motor stopped and relaxation allowed.
Added 1.0 mgm. G. haemorrhoidalis —No effect.
Added 0.01 mgm. Histamine —Sudden maximal rise.

Horn B. Set up and allowed to relax.
Added 5.0 mgm. G. nasalis —No rise.
Changed Ringer.
Added 0.05 mgm. G. haemorrhoidalis. (=1:1,000,000 of dried larvae) —After a pause of half a minute, there was an oscillating rise to one-fourth maximal.
Allowed to relax.
Added 1.0 mgm. G. haemorrhoidalis —Rise to half maximal, with a return to normal.

Added 5.0 mgm. G. haemorrhoidalis —After short pause a small rise to one fourth maximal.
Added 1.0 mgm. G. haemorrhoidalis —No effect.
Hence desensitized to G. haemorrhoidalis.
Added 0.025 mgm. Histamine —Maximal contraction.

Discussion.—This experiment is very similar to the Duck and Hen Egg albumin experiment. While the uterus possesses good sensitiveness towards the non-specific, allied protein, desensitization to this leaves good sensitiveness towards the specific antigen.

But if the uterus is desensitized to the specific antigen first, no sensitiveness is left to the non-specific protein.

We see the close relationship between these two species of *Gastrophilus* larvae which are externally very similar, being differentiated by arrangement of spines on the segments. The possibility suggests itself that if one were confronted with a *Gastrophilus* larva of doubtful identity one might well be able to establish its identity by this method—i.e., testing its extract against guinea pig uteri sensitized with each of the species of *Gastrophilus* after desensitizing to the non-specific proteins by using extract of some other species.

In the second experiment, the analogy to Dale's experiments with the Egg albumins is still further brought out. In this case, as he often found with the albumins, there was no sensitiveness to the allied antigen, *G. nasalis,* but marked sensitiveness to the specific antigen. In the second horn, a reaction was obtained with a quantity of normal saline extract of dried *G. haemorrhoidalis* larvae corresponding to 1 in a million of the dried larvae.

The difficulty in desensitizing is to be explained by the unfavourably large size of the animal. With young 250 gram pigs, desensitization is usually complete after one dose.

It should be noted that in G.P.O/50 (Horn B.) there was no sensitiveness to even 10·0 mgm. of *Ascaris equi,* nor would such be expected. Shimamura and Fujii (24) state that their Askaron (a toxic principle from Ascarids) is also present in many other worms, and also in *Gastrophilus* larvae.

F. Sensitizing Powers of Gastrophilus equi.

(a) *Preparation of Extract.*—As for the other *Gastrophilus* larvae. Like *G. nasalis,* this parasite will not dry to a pulverisable state, but, even after prolonged exposure to concentrated sulphuric acid and even to phosphorus pentoxide in vacuo, it becomes a somewhat moist substance. On this account, we used 5% saline extracts for working, and used larger sensitizing doses for the guinea pigs.

(b) *Determination of M.N.-S.C.*—50 mgm. in a 50.0 c.c. bath is quite non-stimulating.

(c) *Experiments.*—

G.P. O/206. Wt. 290 grams. Received 38 days before 12.5 mgm. G. equi.

Horn A.

3.39 p.m. Added 25.0 mgm. G. equi —Maximal rise, relaxing slowly and typically by 3.53.
Changed Ringer.
4.3 Added 25.0 mgm. G. equi —No rise.
Changed Ringer.
4.14 Added 5.0 mgm. G. haemorrhoidalis —No rise.
Changed Ringer.
4.22 Added 25.0 mgm. G. nasalis —No rise.
4.25 Added 0.05 mgm. Histamine —Maximal rise.

Horn B.

5.9. p.m. Added 0.15 c.c. Horse Serum—No rise.
5.14 Added 25.0 mgm. G. equi —Maximal rise relaxing by 5.25.
Changed Ringer.
5.29 Added 25.0 mgm. G. equi —A slight rise, but probably not anaphylactic.
Changed Ringer.
5.38 Added 25.0 mgm. G. equi —No rise.
Changed Ringer.
5.43 Added 25.0 mgm. G. nasalis —No rise.
Changed Ringer.
5.49 Added 5.0 mgm. G. haemorrhoidalis —No rise.
5.53 Added 0.05 mgm. Histamine —Maximal rise.

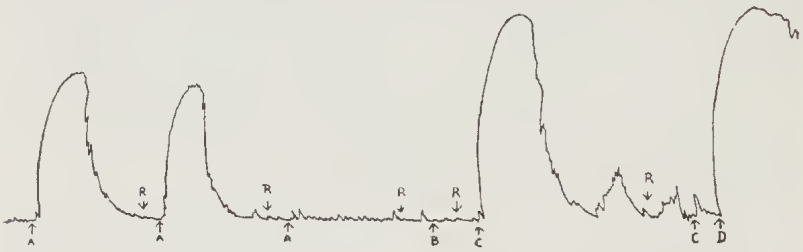

Fig. 1.—Guinea Pig O/206. Weight 290 grams.

Horn B. Received, 38 days before, the normal-saline-soluble proteins in 12.5 mgm. of dried *Gastrophilus equi* larvae.
At "A," 25.0 mgm. of G. equi added; At "B," 25.0 mgm. G. nasalis added; At "C," 5.0 mgm. of G. haemorrhoidalis added; At "D," 0.05 mgm. of Histamine added.
"R" signifies the washing out of the bath with Ringer Solution.

G.P. O/205. Wt. 280 grams. Received 37 days before the Saline-soluble extract of 12.5 mgm. of the dried G. equi.

Horn A.

12.50 p.m. Set up.
1.15 Relaxed
1.16 Added 5.0 mgm. G. haemorrhoidalis —Almost maximal rise, relaxing by 1.25 p.m.
Changed Ringer
1.37 Added 5.0 mgm. G. haemorrhoidalis —Slower, stepping rise to almost maximal, relaxed by 1.43 p.m.
Changed Ringer

1.52	Added 5.0 mgm. G. haemorrhoidalis	—No rise.
	Changed Ringer.	
2.10	Added 25.0 mgm. G. nasalis	—No rise.
	Changed Ringer.	
2.16	Added 25.0 mgm. G. equi	—Rapid rise to maximal, relaxed slowly by 2.31 p.m.
	Changed Ringer.	
2.41	Added 25.0 mgm. G. equi	—No rise.
2.43	Added 0.05 mgm. Histamine	—Maximal rise.

Horn B.

3.37	Added 25.0 mgm. G. nasalis	—No rise.
	Changed Ringer.	
3.45	Added 5.0 mgm. G. haemorrhoidalis	—Almost maximal rise, relaxing by 3.53.
	Changed Ringer.	
4.2	Added 5.0 mgm. G. haemorrhoidalis	—No rise.
	Changed Ringer.	
4.10	Added 5.0 mgm. G. haemorrhoidalis	—No rise.
	Changed Ringer.	
4.47	Added 25.0 mgm. G. equi	—Maximal rise, relaxing by 4.55 p.m.
	Changed Ringer.	
4.59	Added 25.0 mgm. G. equi	—No rise.
5.3	Added 0.05 mgm. Histamine	—Maximal rise.

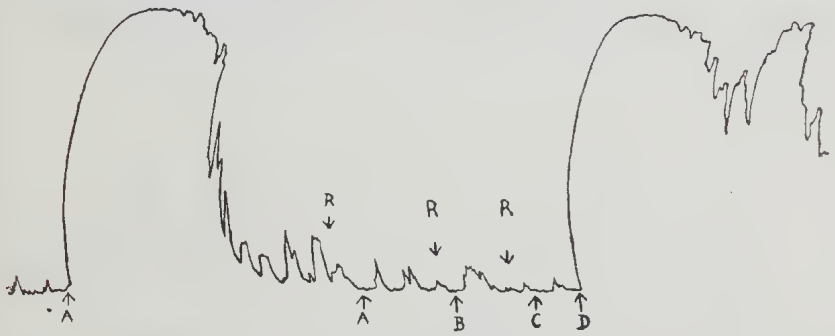

Fig. 2.—Guinea Pig O/205. Weight 280 grams.

Horn A. Received, 37 days before, the normal-saline-soluble proteins in 12.5 mgm. of dried *Gastrophilus equi* larvae.

At "A," 5.0 mgm. of G. haemorrhoidalis added. R signifies the washing out of the bath with Ringer Solution; At "B," 25.0 mgm. of G. nasalis added; At "C," 25.0 mgm. of G. equi added; At "D," 0.05 mgm. of Histamine added.

G. Sensitizing Powers of Fasciola hepatica.

Extract.—Flukes were obtained from livers of sheep, and were well washed with saline. They were then cut up in a petrie dish and placed in the incubator overnight, and next morning they had become a dark thin liquid as though the worms had been dissolved. As autolytic effects were feared the rest of the drying was done over calcium chloride, and concentrated sulphuric acid

in vacuo. It dried to a greyish brown substance which readily powdered. A 1% saline extract was used.

Determination of M.N.-S.C.—This was fixed at 1.6666 (7.5 mgm. in the 50.0 c.c. bath). The dose used to test for sensitiveness was fixed at 5.0 mgm. in the 50.0 c.c. bath=a concentration of 1 : 10,000.

Test.—G.P. O/108. Received 13 days before 5.0 mgm. of dried F. hepatica subcutaneously. Wt. 260 grams.

Horn B. Set up.

Relaxed.	
Added 1.0 c.c. Sheep Serum	—No effect.
Added 5.0 mgm. F. hepatica	—Rise to half maximal.
Relaxed.	
Changed Ringer.	
Added 5.0 mgm. F. hepatica	—No rise.
Added 0.01 mgm. Histamine	—Maximal rise.

G.P. O/109. Received 32 days before 10.0 mgm. F. hepatica powder. Wt. 290 grams.

Added 1.0 Sheep Serum	—No rise.
Added 1.0 mgm. F. hepatica	—After a pause of half a minute, got rise to ½ maximal.
Relaxed.	
Added 2.0 mgm. F. hepatica	—No response.
Added 0.01 mgm. Histamine	—Rise to maximal.

Hence in the above experiments we see that *F. hepatica* has sensitizing powers, and that a reaction was obtained at a concentration of 1: 50,000 of the dry powder (extracted).

Conclusions.

1. Protein-containing extracts of parasites can sensitize the uterus of guinea pigs when injected subcutaneously.
2. Such sensitization is strictly specific, though a peculiar simultaneous non-specific sensitiveness may exist towards extracts of other related forms.
3. Desensitization of the uterus towards the non-specific extract still leaves the muscle sensitive to the extract used for sensitizing the guinea pig.
4. Desensitization towards the specific extract abolishes sensitiveness towards the non-specific extract.
5. The above phenomenon is analogous with that of agglutinin or precipitin action.
6. The sensitized uterus reaction suggests itself as a means whereby mutilated parasites might be identified, or whereby the supposed identity of any two parasitic forms might be tested.

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